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Imaging centromere-based incompatibilities: Insights into the mechanism of incompatibility mediated by low-copy number plasmids

Roxanne Diaz [a,b], Jérôme Rech [a,b], Jean-Yves Bouet [a,b,*]

[a] Laboratoire de Microbiologie et Génétique Moléculaires, Centre National pour la Recherche Scientifique, F-31000 Toulouse, France
[b] Laboratoire de Microbiologie et Génétique Moléculaires, Université de Toulouse, UPS, F-31000 Toulouse, France

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ABSTRACT

In bacteria, low-copy number plasmids are faithfully segregated at cell division by active partition systems that rely on plasmid-specific centromere sequences. When an identical centromere is present on a second plasmid, faithful partition is impaired causing plasmid loss. Depending on the copy number of the co-resident replicon, several mechanisms have been proposed to account for this centromere-based plasmid incompatibility. To gain further insights into these mechanisms, we analyzed the positioning of the F plasmid in the presence of incompatible low- and high-copy number plasmids carrying the F centromere. Our data are fully compatible with the titration hypothesis when extra-centromeres are present on high-copy number plasmids. Interestingly, our plasmids' localization data revealed that the strong incompatibility phenotype, observed when extra centromeres are present on a partition defective low-copy number plasmid, does not directly result from a partition deficiency as previously proposed. We provide a new and simple hypothesis for explaining the strong incompatibility phenotype based on the timing of replication of low-copy number plasmids.

1. Introduction

The survival of low-copy number plasmids in bacteria relies on faithful inheritance to daughter cells (Fig. 1A). This is mainly ensured by finely tuned replication and partition mechanisms (Bouet et al., 2014; del Solar et al., 1998). In the event that plasmids residing within the same bacteria share functional equivalents of either their replication or partition machinery, efficiency of inheritance drastically decreases. This phenomenon, termed plasmid incompatibility, has been studied for years to gain insight into the inner workings of these plasmid maintenance mechanisms (Novick, 1987).

Faithful partition of DNA is ensured by simplistic partition systems that are classified into three main types; types I, II and III (reviewed in Salje, 2010). Type I partition systems are the most widespread throughout low-copy number plasmids and are the only type present on bacterial chromosomes. Type I systems are generically termed ParABS, encoding a Walker-box ATPase, ParA, a DNA binding protein, ParB, and a centromeric sequence, *parS*. A nucleoprotein structure termed the partition complex is formed by the specific binding of ParB to *parS*. ParA is involved in both the separation of duplicated partition complexes and their proper positioning within cells (reviewed in Bouet et al., 2014). The dynamic reactions of the partition components that cooperatively ensure plasmid inheritance have yet to be fully understood (Vecchiarelli et al., 2012).

The F plasmid encodes a type I partition locus, *sopABC* (Ogura and Hiraga, 1983). Three forms of partition-based incompatibility, corresponding to each of the three Sop components, have been described (reviewed in Bouet et al.,

* Corresponding author. LMGM, CNRS, Université Paul Sabatier, Bât. IBCG, 118 route de Narbonne, 31062 Toulouse Cedex 9, France. Fax: +33 561 335 886.
E-mail address: jean-yves.bouet@ibcg.biotoul.fr (J.-Y. Bouet).

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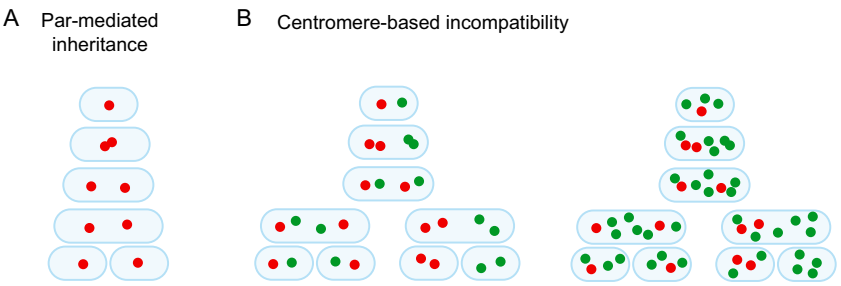

Fig. 1. Partition-mediated incompatibility interferes with faithful inheritance of plasmids. For simplicity, in each case it is assumed that newborn cells contained a single copy of each low-copy number plasmid. (A) Low-copy number plasmids (red circles) rely on plasmid-encoded partition systems to ensure their faithful inheritance to daughter cells (blue oblongs). Type I partition systems are composed of three elements: two genes, *parA* and *parB* encoding for a Walker ATPase and a centromere-binding protein, respectively, and a *cis*-acting centromere site, *parS*. (B) Proposed mechanisms for centromere-based incompatibility depend on plasmid copy-number. (Left) For low-copy number plasmids carrying identical centromere sequences (red and green circles), random positioning of pure plasmid clusters leads to mutual exclusion of the two distinct plasmids in a fraction of the population. (Right) For high-copy number plasmids (green circles), titration of the ParB centromere-binding protein from the low-copy number plasmid (red circles) results in the inability to form a fully functional partition complex, which leads to ineffective segregation and its loss in the growing population.

2007). SopA incompatibility, termed IncI, results from an excess of the ATPase that disrupts the organization of the partition complex (Lemonnier et al., 2000). IncG incompatibility is mediated by the centromere binding protein. An excess of SopB initiates a DNA repair pathway involving homologous recombination and results in the formation of plasmid dimers that, if unresolved, reduce the number of individual F copies to be segregated (Bouet et al., 2006; Kusukawa et al., 1987).

The centromeric sequence *sopC* also exerts incompatibility, termed IncD, when present on a co-resident, but otherwise compatible plasmid (illustrated in Fig. 1B). Previous analyses have shown that the level of incompatibility is mainly correlated with the copy-number of the co-resident plasmid (Bouet et al., 2005). F plasmid loss rate rises to that seen with a deficient partition system when in the presence of high-copy number *sopC*-carrying plasmids. In contrast, plasmid incompatibility seen with low-copy number plasmids carrying *sopC* does not induce a uniform effect on plasmid loss. For instance, the presence of *sopC* on a partition defective low-copy number plasmid provokes a higher-than-random loss of the F plasmid ('strong IncD' phenotype), while the same plasmid efficient in partition induces a relatively low loss rate (Bouet et al., 2005). The exact mechanism by which *sopC* imposes incompatibility is not fully understood, and three main hypotheses have been proposed (Bouet et al., 2007; Ebersbach et al., 2005). One assumes that plasmid replicons are randomly paired by their centromeres for successive partition. Regardless if the pair formed is homologous or heterologous (mixed pair), partition continues as normal, resulting in a random loss rate of plasmids (Austin and Nordstrom, 1990; Bouet et al., 2005). A contradictory view suggests that incompatible plasmids randomly position themselves in avoidance of a co-resident plasmid containing the same partition complex (Ebersbach et al., 2005). A third hypothesis proposes that a competition arises for centromere binding proteins in the presence of a large excess of centromeres, which would inhibit the assembly of a functional partition complex on the F plasmid (Bouet et al., 2005).

Here we aimed to clarify the differences between centromere-based incompatibilities by directly imaging the intracellular positioning of the F plasmid. By using dual fluorescent labeling of the mini-F in the presence of co-resident plasmids carrying the F centromere, we were able to visualize and distinguish the effects of *sopC* exerted incompatibility with plasmids whose copy numbers ranged from 1.6 to ~50 per chromosome. We confirmed that the titration of centromere binding proteins mediates IncD when extra centromeres are present on high-copy number plasmids. In the case of low-copy number co-resident plasmids carrying *sopC*, we clearly showed that Sop-mediated partition is fully functional as positioning is not impaired, even for the strong IncD phenotype. Our observations allow us to suggest a new hypothesis accounting for the strong incompatibility phenotype based on the timing of replication of low-copy number plasmids.

2. Materials and methods

2.1. Escherichia coli *strains and growth conditions*

All strains are derivatives of *E.coli* K-12 W1485, and are listed in Table 1. Normal growth cultures were grown at 37 °C in LB with aeration. Cultures for fluorescence microscopy experiments were grown at 30 °C in M9-glycerol (M9 minimal media supplemented with 0.4% glycerol, 0.2% casamino acids, 0.04 mg ml^{-1} thymine, 0.2 mg ml^{-1} leucine). For selective bacterial growth, the following concentrations of antibiotics were used (μg ml^{-1}): chloramphenicol (Cm, 10), spectinomycin (Sp, 20), and ampicillin (Ap, 20 and Ap, 100 for low- and high-copy number plasmids, respectively).

2.2. *Plasmid constructions*

The plasmids used in this study are listed in Table 1. PCR reactions were performed using PrimesSTAR Max (Clontech) and plasmid constructions were completed using the In-Fusion HD Cloning System (Clontech). The mini-F, pJYB234,